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<110> Montelione, Gaetano Das, Kalyan Arnold, Eddy

Ribosomal RNA Methyltransferases Rima: Target Validation and <120> Processes for Developing an Inhibitor Assay and Idenfitication of Candidate Inhibitors

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<150> US 60/482,722

<151> 2003-06-27

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Gly Asp Ser Ala Glu Met Met Gln Ala Arg Arg Ala Phe Leu Asp Ala

Gly His Tyr Gln Pro Leu Arg Asp Ala Ile Val Ala Gln Leu Arg Glu

Arg Leu Asp Asp Lys Ala Thr Ala Val Leu Asp Ile Gly Cys Gly Glu

Gly Tyr Tyr Thr His Ala Phe Ala Asp Ala Leu Pro Glu Ile Thr Thr 100

Phe Gly Leu Asp Val Ser Lys Val Ala Ile Lys Ala Ala Ala Lys Arg

Tyr Pro Gln Val Thr Phe Cys Val Ala Ser Ser His Arg Leu Pro Phe 130 135 140 Ser Asp Thr Ser Met Asp Ala Ile Ile Arg Ile Tyr Ala Pro Cys Lys 145 150 160 Ala Glu Glu Leu Ala Arg Val Val Lys Pro Gly Gly Trp Val Ile Thr $165 \hspace{0.25cm} 170 \hspace{0.25cm} 175$ Ala Thr Pro Gly Pro Arg His Leu Met Glu Leu Lys Gly Leu Ile Tyr 180 185 190 Asn Glu Val His Leu His Ala Pro His Ala Glu Gln Leu Glu Gly Phe 195 200 205 Thr Leu Gln Gln Ser Ala Glu Leu Cys Tyr Pro Met Arg Leu Arg Gly ASP Glu Ala Val Ala Leu Leu Gln Met Thr Pro Phe Ala Trp Arg Ala 225 230 235 240 Lys Pro Glu Val $\mbox{Trp Gln Thr}$ Leu Ala Ala Lys Glu Val Phe $\mbox{Asp Cys}$ 255Gln Thr Asp Phe Asn Ile His Leu Trp Gln Arg Ser Tyr

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Gly His Tyr Gln Pro Leu Arg Asp Ala Val Ile Asn Leu Leu Arg Glu 65 70 75 80

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Asp Asm Pro Glm Asm Asp Ile 275

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Glu Glu Gln Pro Glu Thr Leu Ser Gly Phe Glu Leu Val Glu Glu Cys 210 215 220

Page 5

Lys Leu His Tyr Pro Met Ala Leu Asn Gly Ser Glu Ala Ala Asp Leu 225 230 235 240 Leu Gln Met Thr Pro Phe Ala Trp Arg Ala Ser Glu Asp Phe Lys His 245 250 255 Arg Val Ser Gln Ser Asp Thr Phe Glu Cys Glu Ala Asp Phe Met Leu 260 265 270 Arg Val Tyr Arg Arg Lys <210> 5 <211> 270 <212> PRT <213> Pseudomonas putida <400> 5 Met Leu Ala Cys Pro Leu Cys Gln Ala Pro Leu Ser Arg Leu Asp Asn 1 10 15 Gly Val Val Cys Pro Ala Gly His Arg Phe Asp Arg Ala Arg Gln Gly $20 \hspace{0.5cm} 25 \hspace{0.5cm} 30$ Tyr Leu Asn Leu Leu Pro Val Gln His Lys Asn Ser Arg Asp Pro Gly 35 40 45 Asp Asn Gln Ala Met Val Glu Ala Arg Arg Asp Phe Leu Asp Ala Gly $50 \hspace{0.5in} 55 \hspace{0.5in} 60$ His Tyr Ala Pro Val Ala Arg Arg Leu Ala Glu Leu Ala Ala Glu Arg Gln Pro Gly Ala Trp Leu Asp Ile Gly Cys Gly Glu Gly Tyr Tyr Thr 85 90 95 Ala Gln Ile Ala Gln Ala Leu Pro Ala Ala Asp Gly Tyr Ala Leu Asp $100 \hspace{1.5cm} 105 \hspace{1.5cm} 110$ Ile Ser Arg Glu Ala Val Lys Arg Ala Cys Arg Arg Ala Ser Ala Val Thr Trp Met Val Ala Ser Met Ala Arg Val Pro Leu Thr Asp Ala Ser 130 135 140 Cys Gln Phe Ile Ala Ser Val Phe Ser Pro Leu Asp Trp Ala Glu Ala 145 150 155 160

Lys Arg Leu Leu Ser Pro Gly Gly Gly Leu Met Arg Val Gly Pro Thr Ser Gly His Leu Met Glu Leu Arg Glu Val Leu Tyr Asp Glu Val Arg $180 \ 185 \ 190$ Pro Tyr Ala Asp Asp Lys His Leu Ala Leu Val Pro Glu Gly Met Ala 195 200 205 His Ala His Ser Glu Thr Leu Glu Phe Arg Leu Ser Leu Ala Ala Pro 210 215 220 Lys Ala Arg Ala Asp Leu Leu Ala Met Thr Pro His Gly Trp Arg Ala 225 230 235 240 Ser Ala Glu Lys Arg Ala Arg Val Ile Asp Gln Pro Glu Pro Phe Glu 245 250 255 Val Thr Val Ser Met Arg Tyr Asp Tyr Phe Val Arg Gln Asp 260 265 270

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Ile Ser Arg Glu Ala Val Lys Arg Ala Cys Arg Arg Ala Pro Gln Leu Thr Trp Leu Val Ala Ser Met Ala Arg Leu Pro Leu Ala Glu Ala Ser 130 135 140 Cys Glu Leu Ile Ala Ser Val Phe Ser Pro Ile Asp Trp Asn Glu Ala 145 150 155 160 Val Arg Val Leu Ala Pro Gly Gly Gly Val Leu Arg Leu Gly Pro Ala 165 170 175 Ser Ala His Leu Leu Glu Leu Arg Gln Arg Leu Tyr Asp Asp Val Arg 180 185 190 Asp Tyr Ala Asp Asp Lys His Leu Ala Gly Leu Pro Ala Pro Leu Ser 195 200 205 Leu Arg His Thr Glu Thr Leu Ser Phe Arg Leu Ala Leu Asp Ser Tyr 210 215 220 Glu Ala Arg Glu Asn Leu Leu Ala Met Thr Pro His Gly Trp Arg Val 225 230 235 240

Val Ala Val Arg Tyr Asp Trp Leu Gln Arg Asp 260 265

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165 170 175 Gly Thr Leu Leu Val Val Thr Pro Gln Gln Asp His Leu Ala Glu Leu 180 185 190 Val Asp Ala Leu Gly Leu Leu Arg Val Arg Asp His Lys Glu Gly Arg 195 200 205 Leu Ala Glu Gln Leu Ala Pro His Phe Glu Ala Val Gly Gln Glu Arg 210 215 220 Leu Arg Thr Thr Leu Arg Leu Asp His Asp Ala Leu Gly Arg Val Val 225 230 235 240 Ala Met Gly Pro Ser Ser Trp His Gln Asp Pro Asp Glu Leu Ala Arg 245 250 255 Arg Ile Ala Glu Leu Pro Gly Ile His Glu Val Thr Leu Ser Val Thr 260 265 270 Phe Thr Val Cys Arg Pro Leu Pro 275 280

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<213> Bacillus subtilis

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Lys Asp Arg Val Ser Leu Leu Lys Glu Met Lys Ser Ala Asp Ile Thr

Val Asp Val Asp Ile Leu Ile Gly Met Lys 275 280

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Gly Tyr Val Asn Phe Leu Gln Thr Lys Ala Asp Thr Glu His Tyr Thr 50 60

Arg Lys Met Phe Glu Pro Arg Arg Arg Leu Ile Gln Ala Gly Met Tyr 65 70 75 80

Gln Asn Leu Leu Thr Glu Ile Gln Lys Ser Phe Val Ser Gly Asn Leu 85 90 95

Leu Asp Val Gly Thr Gly Glu Gly Ser Phe Leu Glu Leu Leu Glu Gly $100 ext{ } 105 ext{ } 110$

Ala Gly Ala Lys Phe Ala Phe Asp Ile Ala Lys Asp Gly Ile Glu Met 115 120 125

Ala Thr Glu Leu Asp Thr Glu Ser Phe Leu Ser Leu Ala Asp Leu Thr 130 140

Asn Leu Pro Phe Ala Asp Glu Ser Leu Ser Val Ile Leu Asn Ile Phe 145 150 155 160

Thr Pro Ser Asn Tyr Ala Glu Phe His Arg Val Leu Thr Glu Asn Gly 165 170 175

Arg Val Ile Lys Ile Ile Pro Asp Arg Asn Tyr Leu His Glu Leu Arg Page 11

Glu Val Tyr Gln Leu Pro Val Asp Tyr Asp Asn Gln Ala Val Ile Glu
Arg Phe Lys Glu Glu Phe Pro Lys Asn Thr Gln Gln Thr Ile Asp Tyr
Thr Phe Glu Ile Pro Glu Asn Leu Arg Gln Asp Phe Leu Leu Met Ser
225 Pro Leu Glu Trp Ser Val Ser Glu Glu Arg Lys Lys Phe Ala Lys Glu
Asn Pro Pro Lys Thr Ala Arg Ile His Val Gln Ile Leu Ile Gly Ile

Lys

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Ala Phe Ala Cys Pro Ile Cys Gln Glu Asn Leu Thr Leu Leu Glu Thr $20 \hspace{0.5cm} 25 \hspace{0.5cm} 30$

Asn Phe Lys Cys Cys Asn Arg His Ser Phe Asp Leu Ala Lys Phe Gly

Tyr Val Asn Leu Val Pro Gln Ile Lys Gln Ser Ala Asn Tyr Asp Lys $50 \hspace{1cm} 60$

Glu Asn Phe Gln Asn Arg Gln Gln Ile Leu Glu Ala Gly Phe Tyr Gln 65 $\,$ 70 $\,$ 75 $\,$

Ala Ile Leu Asp Ala Val Ser Asp Leu Leu Ala Ser Ser Lys Thr Thr $85 \hspace{0.25cm} 90 \hspace{0.25cm} 95$

Thr Thr Ile Leu Asp Ile Gly Cys Gly Glu Gly Phe Tyr Ser Arg Lys $100 \hspace{1cm} 105 \hspace{1cm} 110$

Leu Gln Glu Ser His Ser Glu Lys Thr Phe Tyr Ala Phe Asp Ile Ser Page 12

Lys Asp Ser Val Gln Ile Ala Ala Lys Ser Glu Pro Asn Trp Ala Val 130 135 140 Asn Trp Phe Val Gly Asp Leu Ala Arg Leu Pro Ile Lys Asp Ala Asn 145 150 155 160 Met Asp Ile Leu Leu Asp Ile Phe Ser Pro Ala Asn Tyr Gly Glu Phe 165 170 175 Arg Arg Val Leu Ser Lys Asp Gly Ile Leu Ile Lys Val Ile Pro Thr 180 185 190 Glu Asn His Leu Lys Glu Ile Arg Gln Arg Val Gln Asp Gln Leu Thr Asn Lys Glu Tyr Ser Asn Gln Asp Ile Lys Glu His Phe Gln Glu His 210 215 220Phe Thr Ile Leu Ser Ser Gln Thr Ala Ser Leu Thr Lys Thr Ile Thr 225 230 235 240 Ala Glu Gln Leu Gln Ala Leu Leu Ser Met Thr Pro Leu Leu Phe His 245 250 255 Val Asp Gln Ser Lys Ile Asp Trp Ser Gln Leu Thr Glu Ile Thr Ile 260 265 270 Glu Ala Glu Ile Leu Val Gly Lys Ala Phe 275 280

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Met Thr Pro

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Ala Phe Ala Asp Ala Leu Pro Glu Ile Thr Thr Phe Gly Leu Asp Val 50 55 60 Ser Lys Val Ala Ile Lys Ala Ala Ala Lys Arg Tyr Pro Gln Val Thr

Phe Cys Val Ala Ser Ser His Arg Leu Pro Phe Ser Asp Thr Ser Met

ASP Ala Ile Ile Arg Ile Tyr Ala Pro Cys Lys Ala Glu Glu Leu Ala 100 105 110

Arg Val Val Lys Pro Gly Gly Trp Val Ile Thr Ala Thr Pro Gly Pro 115 120 125

Arg His Leu Met Glu Leu Lys Gly Leu Ile Tyr Asn Glu Val His Leu $130 \hspace{1cm} 135 \hspace{1cm} 140$

His Ala Pro His Ala Glu Gln Leu Glu Gly Phe Thr Leu Gln Gln Ser 145 150 155 160

Ala Glu Leu Cys Tyr Pro Met Arg Leu Arg Gly Asp Glu Ala Val Ala 165 170 175

Leu Leu Gln Met Thr Pro Phe Ala Trp Arg Ala Lys Pro Glu Val Trp 180 185 190

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